Application of MALDI-TOF-MS and FTIR-ATR for *Bacillus pumilus* group species differentiation

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Bacillus pumilus group members, which encompass the closely related *Bacillus pumilus*, *Bacillus safensis, Bacillus altitudinis, Bacillus xiamenensis* and the newly described *Bacillus invictae*, possess a high medical, biotechnological and industrial relevance. Moreover, the difficulty to differentiate this closely related species by conventional and rDNA 16S sequencing methodologies, underscores the need for reliable, easy and rapid methodologies for their correct identification.

In this study we demonstrated the suitability of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) to accurately and rapidly identify the five closely related species enclosed within *B. pumilus* group.

MALDI-TOF-MS and FTIR-ATR were applied to discriminate a clonally diverse collection comprising *B. pumilus* (n=5), *B. safensis* (n=19), *B. altitudinis* (n=1), *B. xiamenensis* (n=1) and *B. invictae* (n=5). Snapshots of different protein composition were acquired with a MALDI-TOF/TOF mass spectrometer, operating in linear positive mode, within the ion range at m/z 2000–12000 and using α-cyano-4-hydroxycinnamic-acid as a matrix. Moreover, the whole-cell content spectra were acquired using a FTIR-ATR System with a PIKE accessory, between 4000 and 400 cm-1, with a resolution of 4 cm-1 and 32 scan co-additions. Both, mass and infrared spectra were analyzed by multivariate data analysis.

The resulting unique protein profile and the differences gathered in the carbohydrates and phospholipids/DNA/RNA vibration regions obtained by these high throughput approaches, proved that MALDI-TOF-MS and FTIR-ATR are valuable tools for *B. pumilus* group species discrimination, and thus, should be promptly considered for these species identification.